

## REMARKS

The Applicants appreciate the Examiner's thorough examination of the subject application. Applicants request reconsideration of the subject application based on the following remarks.

Claims 8, 10-13, 15-18, 20-22 are pending, claims 9, 14, and 19 have been cancelled, and claims 8, 13, 18, and 22 have been amended. Support for the amendments to claims 8, 13, and 18 can be found throughout the specification. Claim 10 has been amended to correct a minor grammatical error and claim 22 was amended to represent language inadvertently deleted in the prior amendment. Support for the language "irradiating the treated sperms with UV light to form a crosslink between a DNA double helix and the psoralen derivative" and "having a small deletion of a plurality base pairs around the crosslinked site in a genome" is found in the specification at page 6 line 2 from the bottom of the page to page 7, line 4. No new matter has been added by virtue of these amendments.

The specification recites at page 6 line 2 from the bottom of the page to page 7, line 4 that small multiple base-pair deletions from the DNA double strand of a genome can be created by UV irradiation of biological organisms which have been pre-treated with a psoralen derivative, e.g., TMP (4-5'-8-trimethylpsoralen). Thus, the instant amendments to the claims are fully supported by the application as originally filed.

Claims 8, 10-13, 15-18, and 20-22 were rejected under 35 U.S.C. §112, second paragraph, as being allegedly indefinite for failing to particular point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 8, 13, and 18, as amended, comply with all the requirements of 35 U.S.C. §112, including the requirements of §112, second paragraph. Claims 10-13, 15-17, and 20-22 depend from claims 8, 13, and 18, and thus also satisfy the requirements of 35 U.S.C. §112, including the requirements of §112, second paragraph.

Withdrawal of the §112, second paragraph, rejections is thus requested.

Claims 8, 10-13, 15-18, and 20-22 were rejected under 35 U.S.C §103 (a) as being allegedly anticipated by Chakrabarti (*Genetics*, 1983, 103:109-123), Grunwald #1 (*Genet. Res.*, 1991, 59:93-101), Grunwald #2 (*Genet. Res.*, 1001, 59:103-116) taken with Thomas (*Mol. Cell. Biol.*, 1996 16(5):2537-2544).

The rejection is traversed.

Claims 8, 13, and 18, as amended, provide methods of mutating a gene in a vertebrate animal and/or analyzing a function of a gene of a vertebrate animal in which a gene is mutated by effecting a small, multiple base-pair deletion.

The present invention provides an efficient method of mutating a gene of vertebrates and analyzing the mutated gene. Applicants have surprisingly discovered that psoralen derivatives causes a mutation in a vertebrate's genome upon exposure to UV-light. More particularly, Applicants have discovered that irradiating a mixture of DNA and a psoralen derivative with UV light induces a small deletion of a plurality of base-pairs in the vertebrate's genome. Applicants have further succeeded in cloning the mutated gene by utilizing the small deletion as a marker

None of the cited references alone or in combination teach or suggest the methods of the invention presented in the instant amendment.

In contrast, none of Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. teach or suggest mutating a gene by incorporating a **small deletion in vertebrate's genome** or cloning such a mutated gene by the methods provided by the invention.

As the reference is understood, Chakrabarti et al. recites that "several observation reported here suggested that an appreciable fraction of  $\gamma$ -ray-induced zebrafish mutations are

**long deficiencies**” (See page 120 line 6-8 in Chakrabarti et al., emphasis added). Thus, the long genome deficiencies induced by exposure to  $\gamma$ -ray irradiation taught by Chakrabarti make it difficult to isolate a mutation at a level of single gene locus.

Grunwald et al. #1 teaches mutation methods using UV light which induce **point mutations**, frameshift mutations, but rarely large deletions or mutations (See page 93, right column, line13-15).

Grunwald et al. #2 recites that “ENU-induced mutations are probably **point mutations**” (See page 115, paragraph(iv) ). Applicants note that cloning of point mutated genes typically requires laborious procedures such as genetic mapping and chromosomal walking.

In contrast, the present invention provides methods of gene mutation in which a **small deletion of a plurality of base-pairs in vertebrate genome** is generated by irradiation of sperm cells contacted with a psoralen derivative with UV light. Psoralen derivatives are DNA cross-linking agents which induce small deletions in vertebrate genome by inducing nucleotide recombinational repair or nucleotide excision repair in the crosslinked interstrand site of DNA. The small deletion in vertebrate genome can be utilized as a marker for cloning the mutated gene.

As discussed in the specification, Applicants have successfully cloned the mutated gene (edw gene) by Representational Difference Analysis (RDA) in which the small deletion is utilized as a marker (See page 15 line 6 to page 16 line 5 in the present specification). Thus, the present method of gene mutation comprising irradiating sperm in presence of a psoralen derivative with UV light has surprising advantages when compared to methods suggested by the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2.

Thomas, et al, fails to overcome the limitations of the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2.

As the reference is understood, Thomas, et al., recites that irradiation of 4'-hydroxymethyl-4,5',8-trimethylpsoralen with near UV-light can induce **point mutation** in DNA found in human cell extracts. That is, Thomas recites that damage dependent replication errors T·A→C·G transitions, transversions at C·G base pairs, and deletions of single A·T base pairs are observed when DNA is exposed to 4'-hydroxymethyl-4,5',8-trimethylpsoralen and near UV-light. Thomas neither teaches nor suggests that small deletions in a vertebrate genome caused by induced by psoralen derivative and UV irradiation.

Moreover, one skilled in the art would not have been motivated by the Thomas teachings to select psoralen derivatives as a mutagen suitable for mutagenesis of vertebrate sperm in view of the variety of known mutagens available in the art.


Accordingly, the present invention would not be obvious to anyone skilled in the art from any of Chalrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 taken with Thomas et al. Applicants request withdrawal of the rejection and reconsideration of the claims.

Reconsideration and withdrawal of the rejection of the noted claims are thus requested.

It is believed the application is in condition for immediate allowance, which action is earnestly solicited.

Respectfully submitted,

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